

WHAT IS CLAIMED IS:

1. A translation system comprising:  
an orthogonal lysyl tRNA (lysyl O-tRNA) or modified variant thereof;  
an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially charges an orthogonal lysyl tRNA or modified variant thereof with one or more amino acid; or  
an orthogonal lysyl tRNA (lysyl O-tRNA) or modified variant thereof and an orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially charges the lysyl O-tRNA or modified variant thereof with one or more amino acid.
2. The translation system of claim 1, wherein the translation system comprises a cell.
3. The translation system of claim 2, wherein the cell is an *E. coli* cell.
4. The translation system of claim 1, wherein the amino acid is an unnatural amino acid.
5. The translation system of claim 4, wherein the unnatural amino acid is homoglutamine.
6. The translation system of claim 1, wherein the lysyl O-tRNA or modified variant thereof, the O-RS, or both, are derived from *Pyrococcus horikoshii* (PhKRS).
7. The translation system of claim 6, wherein the O-RS is PhKRS, E444G, PhΔAD, or an I41 and/or S268 mutant of PhΔAD.
8. The translation system of claim 6, wherein the O-RS, when expressed in an *E. coli* cell displays a toxicity that is the same as or less than an I41 and/or S268 mutant of PhΔAD.
9. The translation system of claim 1, wherein the lysyl O-tRNA or modified variant thereof comprises a recognition sequence for a four base codon or an amber codon.
10. The translation system of claim 1, wherein the lysyl O-tRNA or modified variant thereof comprises a recognition sequence for AGGA.
11. The translation system of claim 1, wherein the lysyl O-tRNA or modified variant thereof comprises an anti-codon loop comprising a CU(X)<sub>n</sub>XXXAA sequence.

12. The translation system of claim 11, wherein the CU(X)<sub>n</sub>XXXAA sequence comprises CUCUAAA or CUUCCUAA.
13. The translation system of claim 1, wherein the lysyl O-tRNA or modified variant thereof comprises SEQ ID NO:24 or SEQ ID NO:26.
14. The translation system of claim 1, wherein the O-RS and lysyl O-tRNA or modified variant thereof are at least 50% as effective at suppressing a stop or frame shift selector codon as E444G, PhΔAD, or an I41 and/or S268 mutant of PhΔAD, in combination with an O-tRNA of SEQ ID NO:24 or SEQ ID NO:26.
15. The translation system of claim 1, comprising an additional O-RS and an additional O-tRNA, wherein the additional O-RS and the additional O-tRNA suppress a frame shift selector codon that is different from a frame shift selector codon suppressed by the lysyl O-tRNA or variant thereof and the O-RS that preferentially charges the lysyl O-tRNA or modified variant thereof.
16. The translation system of claim 1, wherein the translation system suppresses both a four base selector codon and a stop selector codon in a target nucleic acid that encodes a target polypeptide.
17. The translation system of claim 16, wherein the four base selector codon comprises the sequence AGGA and the stop selector codon comprises the sequence TAG or UAG.
18. The translation system of claim 1, comprising a target nucleic acid that comprises a four base selector codon.
19. The translation system of claim 18, comprising a protein encoded by the target nucleic acid.
20. The translation system of claim 19, wherein the protein comprises a homoglutamine.
21. The translation system of claim 1, comprising a target nucleic acid that comprises a four base selector codon and a stop selector codon.
22. The translation system of claim 21, comprising a protein encoded by the target nucleic acid, wherein the protein comprises at least two different unnatural amino acids.

23. A translation system comprising:

a first orthogonal tRNA (O-tRNA) that recognizes a four base selector codon;

a first orthogonal aminoacyl tRNA synthetase (O-RS) that preferentially charges the O-tRNA with a first unnatural amino acid;

a second O-tRNA that recognizes a stop selector codon; and,

a second O-RS that preferentially charges the second O-tRNA with a second unnatural amino acid.

24. The translation system of claim 23, wherein the four base selector codon is AGGA.

25. The translation system of claim 23, wherein the stop codon is UAG.

26. The translation system of claim 23, wherein the translation system comprises a cell.

27. The translation system of claim 23, wherein the first or second O-tRNA is an orthogonal lysyl tRNA (lysyl O-tRNA) or modified variant thereof.

28. The translation system of claim 23, wherein the first O-tRNA is an orthogonal lysyl tRNA (lysyl O-tRNA) or modified variant thereof and the second O-tRNA is an orthogonal tyrosyl tRNA (tyrosyl O-tRNA) or modified variant thereof.

29. The translation system of claim 23, further comprising a nucleic acid comprising at least a four base selector codon and a stop selector codon.

30. The translation system of claim 29, wherein the four base selector codon is AGGA and the stop selector codon is TAG or UAG.

31. The translation system of claim 29, wherein the nucleic acid is an expressed RNA.

32. The translation system of claim 29, wherein the translation system comprises a protein encoded by the nucleic acid, which protein comprises at least two different unnatural amino acids.

33. The translation system of claim 32, wherein the protein comprises a homoglutamine.

34. The translation system of claim 32, wherein the protein is homologous to myoglobin.

35. The translation system of claim 23, comprising homoglutamine.

36. A composition comprising: PhKRS, E444G, Ph $\Delta$ AD, an I41 and/or S268 mutant of Ph $\Delta$ AD, or a conservative variant thereof.

37. A nucleic acid that encodes PhKRS, E444G, Ph $\Delta$ AD, an I41 and/or S268 mutant of Ph $\Delta$ AD, or a conservative variant thereof.

38. A nucleic acid that comprises or encodes a tRNA that corresponds to SEQ ID NO:24 or SEQ ID NO:26, or a conservative variation thereof.

39. A composition comprising an orthogonal aminoacyl-tRNA synthetase (O-RS), wherein the O-RS preferentially aminoacylates an O-tRNA with a homoglutamine.

40. The composition of claim 39, wherein the O-RS comprises an I41 and/or S268 mutation of Ph $\Delta$ AD, or a conservative variation thereof.

41. The composition of claim 39, wherein the O-RS preferentially aminoacylates the O-tRNA with an efficiency of at least 50% of the efficiency of an I41 and/or S268 mutation of Ph $\Delta$ AD.

42. The composition of claim 39, wherein the O-RS is derived from a *Pyrococcus horikoshii*.

43. The composition of claim 39, comprising the O-tRNA, wherein the O-tRNA recognizes a four base selector codon.

44. The composition of claim 43, wherein the four base selector codon comprises an AGGA sequence.

45. The composition of claim 39, comprising a cell, wherein the O-RS is encoded by one or more nucleic acids in the cell.

46. The composition of claim 45, wherein the cell is an *E. coli* cell.

47. The composition of claim 39, comprising a translation system.

48. The composition of claim 39, comprising a cell, wherein the O-RS is encoded by one or more nucleic acids in the cell, the cell further comprising:

an orthogonal -tRNA ( O-tRNA); and,

a homoglutamine;

wherein the O-tRNA recognizes a first selector codon, and the O-RS preferentially aminoacylates the O-tRNA with the first homoglutamine.

49. The composition of claim 48, wherein the cell comprises a target nucleic acid that encodes a polypeptide of interest, wherein the target nucleic acid comprises a selector codon that is recognized by the O-tRNA.

50. The composition of claim 48, wherein the O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO:24 or SEQ ID NO:26, or a complementary polynucleotide sequence thereof, and wherein the O-RS comprises an amino acid sequence corresponding to E444G, Ph $\Delta$ AD, an I41 and/or S268 mutant of Ph $\Delta$ AD, or a conservative variation thereof.

51. The composition of claim 48, wherein the O-RS and O-tRNA are at least 50% as effective at suppressing a stop or frame shift selector codon as E444G, Ph $\Delta$ AD, or an I41 and/or S268 mutant of Ph $\Delta$ AD, in combination with an O-tRNA of SEQ ID NO:24 or SEQ ID NO:26.

52. The composition of claim 48, wherein the cell is an *E. coli* cell.

53. The composition of claim 48, wherein the cell further comprises an additional different O-tRNA/O-RS pair and an additional different unnatural amino acid, wherein the O-tRNA recognizes a second selector codon and the O-RS preferentially aminoacylates the O-tRNA with the second unnatural amino acid.

54. The composition of claim 53, wherein the cell comprises a target nucleic acid that comprises the first and second selector codons.

55. The composition of claim 54, wherein the cell comprises a protein encoded by the target nucleic acid, which protein comprises at least two different unnatural amino acids.

56. A composition comprising a protein, wherein the protein comprises a homoglutamine.

57. The composition of claim 56, wherein the protein comprises an amino acid sequence that is at least 75% identical to that of a wild-type therapeutic protein, a diagnostic protein, an industrial enzyme, or portion thereof.

58. The composition of claim 56, wherein the composition comprises a pharmaceutically acceptable carrier.

59. A method for selecting an active orthogonal-aminoacyl-tRNA synthetase (O-RS) that loads a homoglutamine on an orthogonal tRNA (O-tRNA), the method comprising: subjecting a population of cells to selection, wherein the cells collectively comprise:

1) the O-tRNA, wherein the O-tRNA is orthogonal to members of the population of cells that comprise the O-tRNA;

2) a plurality of O-RSs that comprise one or more active O-RS members that load the O-tRNA with a homoglutamine in one or more cells of the population;

3) a polynucleotide that encodes a selectable marker, wherein the polynucleotide comprises at least one selector codon that is recognized by the O-tRNA; and,

4) homoglutamine;

wherein a target cell in the population that comprises the active O-RS is identified by an enhanced suppression efficiency of the selectable marker as compared to a suppression efficiency of a control cell lacking the plurality of RSs but comprising the O-tRNA; and,

selecting the target cell, thereby selecting the active O-RS.

60. The method of claim 59, wherein the cells are additionally selected to eliminate cells that comprise a non-target O-RS that charges the O-tRNA with an amino acid other than homoglutamine.

61. The method of claim 59, wherein the selection comprises a positive selection and the selectable marker comprises a positive selection marker.

62. The method of claim 59, wherein the plurality of RSs comprise mutant RSs, RSs derived from one or more species other than the first species or both mutant RSs and RSs derived from a species other than the first species.

63. An orthogonal aminoacyl-tRNA synthetase identified by the method of claim 59.

64. A method of producing a protein in a cell with a homoglutamine at a specified position, the method comprising:

growing, in an appropriate medium, the cell, where the cell comprises a nucleic acid that comprises at least one selector codon and encodes a protein; and,  
providing the homoglutamine;  
wherein the cell further comprises:  
an orthogonal -tRNA (O-tRNA) that recognizes the selector codon; and,  
an orthogonal aminoacyl-tRNA synthetase (O-RS) that preferentially aminoacylates the O-tRNA with the homoglutamine; and,  
incorporating the homoglutamine into the specified position in response to the selector codon, thereby producing the protein.

65. The method of claim 64, wherein the O-RS comprises a amino acid sequence corresponding to E444G, Ph $\Delta$ AD, an I41 and/or S268 mutant of Ph $\Delta$ AD, or a conservative variation thereof.